RESEARCH ARTICLE

Cyanobacterial diversity in Salar de Huasco, a high altitude saline wetland in northern Chile: an example of geographical dispersion?

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Abstract

The diversity of *Cyanobacteria* in water and sediment samples from four representative sites of the Salar de Huasco was examined using denaturing gradient gel electrophoresis and analysis of clone libraries of 16S rRNA gene PCR products. Salar de Huasco is a high altitude (3800 m altitude) saline wetland located in the Chilean Altiplano. We analyzed samples from a tributary stream (H0) and three shallow lagoons (H1, H4, H6) that contrasted in their physicochemical conditions and associated biota. Seventy-eight phylotypes were identified in a total of 268 clonal sequences deriving from seven clone libraries of water and sediment samples. *Oscillatoriales* were frequently found in water samples from sites H0, H1 and H4 and in sediment samples from sites H1 and H4. *Pleurocapsales* were found only at site H0, while *Chroococcales* were recovered from sediment samples of sites H0 and H1, and from water samples of site H1. *Nostocales* were found in sediment samples from sites H1 and H4, and water samples from site H1 and were largely represented by sequences highly similar to *Nodularia spumigena*. We suggest that cyanobacterial communities from Salar de Huasco are unique – they include sequences related to others previously described from the Antarctic, along with others from diverse, but less extreme environments.

Introduction

The statement of the Dutch microbiologist Bass-Becking '*everything is everywhere*, but *the environment selects*' (1934) is frequently used as the starting point of many studies on prokaryotic and protist biodiversity and biogeography (de Wit & Bouvier, 2006). '*Everything is everywhere*' reflects the concept that all microorganisms are cosmopolitan and statement '*the environment selects*' implies that specific microorganisms are observed in their characteristic environments. The statement of Bass-Becking is now under critical review.

Recent evidence indicates the presence of possible endemism in prokaryotes. Evidence for endemism is largely restricted to samples from extreme environments, for example *Synechococcus* inhabiting mats in hot springs (Papke *et al.*, 2003) and in *Sulfolobus solfataricus* (Whitaker *et al.*, 2003).

The literature includes considerable support for the cosmopolitan distribution of prokaryotes, due to their high dispersion capacity, the enormous size of microbial populations and the low probability of extinction (Fenchel, 2003). However, estimates of the scope for their distribution are influenced by the level of taxonomic resolution applied and the technique used to identify them. For example, it is well accepted that Bacteria and Archaea are globally distributed (using 16S rRNA gene sequences) (DeLong & Pace, 2001) but at lower taxonomic levels (e.g. genus level) prokaryotes have a cosmopolitan distribution in their respective habitats (Ramette & Tiedje, 2006).

Although evidence for potential endemism among *Cyanobacteria* is growing, based on morphological studies, endemism of *Cyanobacteria* in Antarctic habitats was discarded and *Cyanobacteria* was considered as having cosmopolitan distribution (Vincent, 2000; Taton *et al.*, 2003). Conversely, molecular tools have revealed evidence for a bipolar distribution of Antarctic and Arctic *Cyanobacteria* (Comte *et al.*, 2007), and the existence of some clusters that appear endemic for Antarctica (e.g. Taton *et al.*, 2006a; Laybourn-Parry & Pearce, 2007).

In terms of their morphology and phylogenetics, *Cyanobacteria* are one of the most diverse groups of prokaryotes (Waterbury, 2006). Their ecological tolerance (e.g. to a broad range of temperatures, high salinities, adaptations to light) contributes to their competitive success in a variety of environments, both as planktonic or benthic organisms (Badger *et al.*, 2006; Cohen & Gurevitz, 2006). *Cyanobacteria* can dominate primary production in some environments including microbial mats (Stal, 1995) and some extreme environments, such as Antarctic permafrost aquatic systems (Jungblut *et al.*, 2005).

Cyanobacteria are currently placed into five orders: *Chroococcales*, *Pleurocapsales*, *Oscillatoriales*, *Nostocales* and *Stigonematales* (e.g. Tomitani *et al.*, 2006). Members of the *Chroococcales* and *Oscillatoriales* are dispersed throughout the phylogenetic tree, indicating that these two orders at least do not represent coherent evolutionary lineages (Waterbury, 2006). Recent studies in wetlands located in the Chilean Altiplano described high microbial diversity and high spatial variability of the microbial communities (Demergasso *et al.*, 2004). The athalassohaline water bodies located in this area are subject to extreme conditions including high UV radiation, low temperatures, negative water balance and variable salt concentration. Little information is available on cyanobacterial diversity in Andean salares, with the exception of a study examining the microbial mats of the Salar de Llamará, located in the Atacama Desert (Demergasso *et al.*, 2003). This study revealed the presence of *Cyanothece* sp., *Synechococcus* sp., *Microcoleus* sp., *Oscillatoria* sp., *Gloeocapsa* sp. and *Gloeobacter* sp. in different mats. *Oscillatoria* sp. was also revealed to be a dominant component of the cyanobacterial community of the Laguna Tebenquinche in the Salar de Atacama (Zúñiga *et al.*, 1991). In the same region, *Cyanobacteria* have been studied in the high altitude El Tatio hot-springs where *Chroococcidiopsis* sp., *Phormidium* sp. and *Lyngbya* sp. were reported (Fernandez-Turiel *et al.*, 2005; Phoenix *et al.*, 2006). Also, studies of quartz stones from the Atacama Desert showed a predominance of hypolithic *Cyanobacteria* (Warren-Rhodes *et al.*, 2006) and endolithic *Cyanobacteria* in soil gypsum (Dong *et al.*, 2007).

The Salar de Huasco is an Andean salar (Chong, 1984) located at 3800 m altitude and was selected as a model of altiplanic wetlands because it is subject to low anthropogenic perturbations and exhibits visual spatial variability. Using 16S rRNA gene clone libraries and PCR-denaturing gradient gel electrophoresis (DGGE), we examined cyanobacterial community structure in water and sediment samples collected from four different sites within the Salar de Huasco. We also discussed the biogeographical relationships of *Cyanobacteria* found in this almost unexplored habitat and possible connections to other extreme habitats.

Materials and methods

Site description and sampling

Samples of water and sediment from sites H0, H1, H4 and H6 were collected in austral summer (January 2005) in Salar de Huasco (20°18′S, 68°50′W), an athalassohaline, pH – neutral and high altitude (3800 m) wetland located in the Chilean Altiplano. Saline wetlands in the Altiplano (locally called 'salares') are hydrologically active receiving water inputs, particularly rainfall, during the austral summer (Risacher *et al.*, 1999). The wetland was formed during the Pleistocene and evolved into an evaporitic basin, due to high rates of evaporation and erosion (Chong, 1984). The site includes freshwater streams (e.g. site H0), bofedales (local name for peatlands) and permanent lagoons with different salt concentration (e.g. sites H1, H4, H6), and are found in an area of 51 km^2 (Risacher *et al.*, 1999). During our work, total salt concentration ranged from 0.42 to 64.9 $g\,L^{-1}$ (data not shown).

Microscopic observations of water samples

We examined phytoplankton from water samples quantitatively and qualitatively from samples collected in previous sampling trips (September 2002, March 2003, September 2003). After collection, water samples were preserved in lugol for lately identification and analyzed in a 1-mL Sedwick–Rafter chamber via an inverted microscope (Olympus CK2) (Wetzel & Likens, 1991). Phytoplankton was identified according to Liberman & Miranda (1987) and Parra & Bicudo (1995) following Bourelly (1970). Abundance was evaluated as standard units (SU) per liter, and each SU was 400 μm^2 (Sournia, 1978).

DNA extraction and PCR amplifications

Environmental DNA was extracted from water and sediment samples from each site. Water samples were filtered at the site onto 0.2-μm, 25-mm-diameter filters (Supor 200, Pall). The filtered volume varied between 0.05 and 1 L depending on the amount of suspended solids in the samples. Filters and sediment samples were maintained at −20 °C for several days before subsequent DNA extraction in the lab.

Oligonucleotide primers Eub9-27F and Eub1542R (Stackebrandt & Liesack, 1993) were used to PCR-amplify eubacterial 16S rRNA gene. Fragments of cyanobacterial 16S rRNA gene were amplified with a nested PCR approach using PCR products from eubacterial 16S rRNA gene as template and the following set of primers CYA106F, CYA359F, CYA781R(a) and CYA781R(b) (Nübel *et al.*, 1997). Each PCR reaction contained 10 × PCR-buffer with 2 mM $MgCl_2$ (Roche), 200 mM dNTP mixture (Gibco), 1 pmol of each primer,

2.5 U Taq polymerase (Roche), 10–100 ng template DNA and water to a final volume of 50 μL.

DGGE

PCR products were generated with primers P2-P3, and DGGE was performed according to Muyzer *et al.* (1993) in the D Gene System (BioRad) at 60 °C, 200 V for 6 h. The gels were stained with silver nitrate (Sanguinetti *et al.*, 1994). In order to examine relationships between communities in the different samples, a matrix was constructed from the distribution pattern of the bands in different samples, and cluster analyses (WPGMA), based on percent similarity between the samples, were conducted using the MULTIVARIATE statistical package (MSVP version 3.12d; Kovach Computing Services, Wales, UK). Pairwise comparison between samples were made using *t*-test (Rothrock & Garcia-Pichel, 2005).

Cloning and 16S rRNA gene sequence analysis

Cyanobacterial clone libraries were generated from water and sediment samples collected from the four study sites. Purified amplicons were cloned into pCR-Blunt vector (Invitrogen) according to the manufacturer's instructions. Ninety-six clones per sample were picked, and inserts were amplified with M13F/R primers. Cycle sequencing was performed with M13F/R PCR products using the BigDye Terminator Cycle Sequencing Kit v3.1 and analyzed on an automated capillary sequencer (model 3100 Gene Analyzer, Applied Biosystems). Sequences were checked for chimeras using CHIMERA CHECK from RDP II (http://rdp.cme.msu.edu).

Rarefaction curves (Simberlof, 1972) were calculated with the RARFAC program (http://www.icbm.de/pmbio/downlist.htm) and used to evaluate whether sufficient numbers of clones were screened to estimate total diversity in each clone library. The Shannon-Weaver index (H′) was used to estimate diversity of clones according to: $H' = \sum pi(\ln pi)$ where p*i* is the relative abundance of the phylotype *i* (Krebs, 1998). The total number of phylotypes in each clone library was estimated by calculating the nonparametric richness estimator Chao1. Based on the frequency with which different phylotypes occurred, coverage was calculated in order to estimate the proportion of phylotypes in the sample which is represented in the library. These analyses were performed via the web interface available at http://www.aslo.org/lomethods/free/2004/0114a.html (Kemp & Aller, 2004).

Phylogenetic analysis

The 16S rRNA gene sequences were analyzed by BLAST search (http://www.ncbi.nlm.nih.gov/blast) to determine the closest relatives present in the database. Parameters used in the BLAST nucleotide database analysis for each sequence included a low complexity filter, a linear gap cost and 1, 2 match/mismatch scores.

Phylogenetic affiliations were inferred with the classifier tool in RDP II (http://rdp.cme.msu.edu). Sequences were aligned using the alignment tool of the ARB package (http://www.arb-home.de) and we conducted maximum-likelihood analyses in the program PHYML (Guindon *et al.*, 2005) using the GTR substitution model (generalized time reversible) with bootstrap resampling (100 iterations). Topologies of the trees were confirmed with a neighbor-joining tree calculated from a distance matrix by the method of Jukes and Cantor in MEGA3 (Kumar *et al.*, 2004). Sequences not included in the ARB database were obtained from GenBank. Sequences with similarities $> 99\%$ were considered to represent the same phylotype (using a threshold of 97.5% we obtained the same phylotypes) (Hughes *et al.*, 2001). Sequences with $< 93\%$ similarity with cultured relatives were considered as unidentified *Cyanobacteria* (UC) (Taton *et al.*, 2003).

Nucleotide sequence accession numbers

The nucleotide sequences from this study are available in GenBank (http://www.ncbi.nlm.nih.gov) under accession numbers EF632952–EF633026.

Results and discussion

Microscopic observation of water samples

Cyanobacteria occupied a minor component of the phytoplankton community from water samples ($< 30\%$). Diatoms were dominant, particularly at sites H4 and H6, where in some periods all phytoplankton were diatoms (Table 1). In terms of genera richness, diatoms included between eight and 16 genera, green algae one to four and *Cyanobacteria* one to two. The *Cyanobacteria* genera were identified as *Oscillatoria* sp., *Anabaena* sp. and *Spirulina* sp. These genera, except *Spirulina* sp., have been already reported in Salar de Llamará using microscopy (Demergasso *et al.*, 2003).

Composition of cyanobacterial communities in Salar de Huasco

Although diatoms dominated the phytoplankton community of Salar de Huasco, we detected between five and 11 cyanobacterial DGGE bands in water samples and nine to 14 in sediment samples which can be used as richness indicators (e.g. Reche *et al.*, 2005). This reveals an improved ability to detect *Cyanobacteria* using molecular methods compared with more traditional (i.e. direct observation) techniques.

Table 1. Phytoplanktonic composition in four sites of Salar de Huasco

		Diatoms		Green algae		*Cyanobacteria*		
Site	Sampling date	RA (%)	R (n)	RA (%)	R (*n*)	RA (%)	R (*n*)	Genera of cyanobacteria
H0	Sep-02	89.9	8.0	3.5	1.0	6.5	1.0	*Oscillatoria* sp.
	Mar-03	47.7	11.0	46.9	4.0	5.4	1.0	*Oscillatoria* sp.
	Sep-03	71.5	16.0	28.5	1.0	0.0	0.0	
H1	Sep-02	87.2	8.0	8.7	1.0	4.1	2.0	*Anabaena* sp., *Oscillatoria* sp.
	Mar-03	66.3	12.0	3.5	2.0	30.2	1.0	*Oscillatoria* sp.
	Sep-03	97.8	15.0	2.2	1.0	0.0	0.0	
H4	Sep-02	100.0	10.0	0.0	0.0	0.0	0.0	
	Mar-03	96.4	10.0	0.0	0.0	0.0	0.0	
	Sep-03	100.0	13.0	0.0	0.0	0.0	0.0	
H6	Sep-02	100.0	8.0	0.0	0.0	0.0	0.0	
	Mar-03	79.5	11.0	0.0	0.0	20.5	2.0	*Oscillatoria* sp., *Spirulina* sp.
	Sep-03	91.7	14.0	0.0	0.0	8.3	1.0	*Oscillatoria* sp.

RA, relative abundance; R, richness.

Cluster analysis (WPGMA) of DGGE bands was conducted in order to examine similarities in cyanobacterial composition between samples and sites. We found significant differences in the DGGE band pattern between samples: H0s and H0w (*t*-test: $t = 2.87$, d.f. = 44, $P < 0.006$), H0s and H4w (*t*-test: $t = 2.13$, d.f. = 44, $P < 0.038$) and H0s and H6w (*t*-test: $t = 2.49$, d.f. = 44, $P < 0.017$). Samples of water and sediment from site H6 clustered together, but other samples did not show any clear grouping reflecting sample type or site (Fig. 1). The number of DGGE bands and clonal sequence diversity was higher in sediment than in water samples (Table 2), except for the sample H1w.

Cyanobacterial 16S rRNA gene clone library

Four clone libraries of water and sediment (sites H0, H1, H4 and H6) were constructed. From the water samples 161 clones were obtained and grouped into 49 phylotypes. Sequence analysis of clones from sites H4 and H6 revealed a large number of unspecific sequences related to Bacteria (92% of the clones of H4 and 90% of H6). These libraries were subsequently excluded from rarefaction analyses. We obtained 121 clones in 29 phylotypes from sediment samples but obtained no clones from site H6 (Table 2). Rarefaction analysis revealed saturation in all libraries at a number of phylotypes between six and 14, except for the sample H1w (29 phylotypes) (data not shown). In addition, coverage indicated that more than 59% of total diversity was detected in the clone libraries. The richness estimator Chao1 was higher than the number of observed phylotypes in all libraries but almost identical for one sample (H0s). The cyanobacterial community at site H1 was the most diverse and had the highest number of phylotypes (Table 1). A BLAST search was used to find similarities of the phylotypes with sequences in GenBank. Most phylotypes from water samples and sediment samples had a similarity between 98–99% and 96–97%, respectively, with their closest cultured relatives (Fig. 2). Threshold values of 97.5% have frequently been used to distinguish between cyanobacterial species (Taton *et al.*, 2003, 2006b). Because 16S rRNA gene sequences with 97.5% of similarity likely correspond to DNA–DNA hybridization values of $< 70\%$, these sequences probably represent two distinct species (Stackebrandt & Göbel, 1994). If we consider 97.5% as a threshold value, 90% of the sequences from sediments and 59% from water samples could be considered as new phylotypes (Fig. 2).

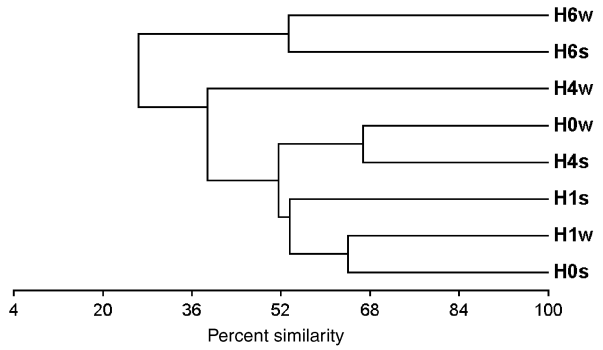

Fig. 1. WPGMA clustering of DGGE band patterns of 16S rRNA gene from water and sediment samples of the four sites in Salar de Huasco.

Phylogenetic diversity

Cyanobacterial communities were distinct an each of the four sites from the Salar de Huasco. The sequences were related to various contrasting environments including phylotypes retrieved from microbial mats of Antarctic, hot springs, river biofilm, and both marine and freshwater environments (Table 3). Most of the sequences from this study, including those from water samples, were related to

Table 2. Summary of data obtained from DGGE and 16S rRNA gene clone libraries

	DGGE		16S rRNA gene clone library				
Sample	Number of bands	Shannon-Weaver diversity index (H′)	Number of clones	Number of phylotypes	Coverage (%)	Chao1	Shannon-Weaver diversity index (H′)
H0w	5	1.61	90	7	95.55	11.13	0.59
H1w	11	2.40	57	29	64.91	67.45	3.01
H4w	7	1.95	7	6	ND	ND	ND
H6w	6	1.79	7	7	ND	ND	ND
H0s	14	2.64	50	5	98.00	5.16	0.90
H1s	8	2.08	44	10	88.63	20.50	1.55
H4s	10	2.30	27	14	59.25	43.26	2.21
H6s	9	2.20	0	0	ND	ND	ND

ND, not determined.

benthic *Cyanobacteria*. In Salar de Huasco, as in others salares, water level varies both on an interannual basis and also between sites. The apparent absence of planktonic *Cyanobacteria* may reflect the low water level in the lagoons (< 10 cm) and the concentration of salt due evaporation are such to allow the establishment of benthic *Cyanobacteria* (Badger *et al.*, 2006).

We detected *Cyanobacteria* from the four orders *Oscillatoriales*, *Nostocales*, *Pleurocapsales*, and *Chroococcales* (Fig. 3). In addition, between 8–18% and 2–19% of unidentified *Cyanobacteria* were found in water and sediment samples, respectively. In detail, the following *Cyanobacteria* were identified at the different sites (Fig. 4): In water samples from site H0, the clone library was dominated by *Oscillatoriales* but *Chroococcales* and *Pleurocapsales* were dominant in sediment samples, *Oscillatoriales* were the abundant group in water and sediment samples from the other sites, and *Pleurocapsales* were only found at H0. *Nostocales* were identified at H1 (water and sediment) and from sediment at H4.

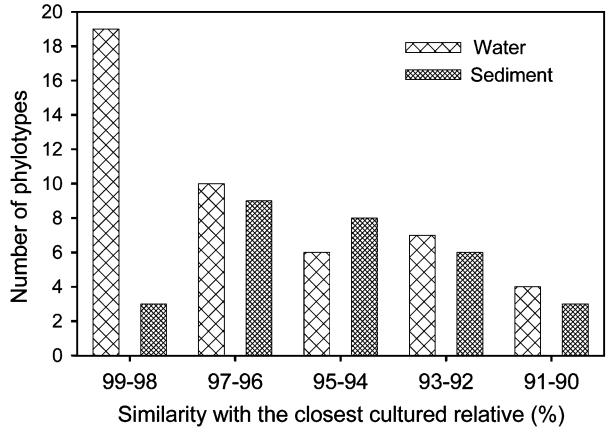

Fig. 2. Percent similarity of the phylotypes with their closest cultured relatives.

Seventy-eight phylotypes, defined to have 99% similarity between the clones, were grouped into 12 clusters with distinct phylogenetic affiliation (Table 3, Fig. 4). Clusters A, B, D, G and H were formed at < 97% similarity with the closest relatives in GenBank (underlined clones). Most sequences with lower similarity with their closest relatives from GenBank were retrieved from the site H1 and were distributed across the 12 defined clusters.

Phylotype H1w-93 had 91% similarity with the planktonic *Limnothrix* sp. (cluster A). Cluster B included the phylotypes H4s-42 and H1w-72 that grouped with the phylotype 16ST17, previously described from Antarctic environments (Taton *et al.*, 2006a) and with the benthic *Geitlerinema carotinosum*. Cluster D included the phylotypes H0s-1 and H1w-7 related to the unicellular *Chamaesiphon subglobosus*. Cluster G included two phylotypes from water samples (site H4) that had 95% similarity with members of the *Chroococcales*. Cluster H consisted of two groups, one clustered with phylotypes from sites H1 and H6 and was distantly related to their first hit in BLAST (< 92%). The second group was formed with two phylotypes from water samples from H1 that showed 95% similarity to *Merismopedia glauca*.

Clusters C, J, K, and L were affiliated to the *Oscillatoriales*, cluster E to the *Nostocales* and cluster F to the *Pleurocapsales* (Fig. 4, Table 3). Cluster C was comprised of phylotypes from sites H0 and H1 that were related to the benthic, filamentous cyanobacterium *Phormidium*. Phylotype H1w-15 had 98% similarity with *Phormidium inundatum* SAG 79.79 isolated from thermal waters in France (Marquardt & Palinska, 2007). A further two phylotypes from water samples (site H0) had 96–99% similarity with the clone Fr147 retrieved from microbial mats of Lake Fryxell in Antarctica (Taton *et al.*, 2003). Three phylotypes from sediment and one from water samples all collected at site H1, clustered together with *Phormidium* sp. ETS.05 (93–99% similarity) previously isolated from thermal springs in Italy (Berrini *et al.*, 2004). Most of the phylotypes

Table 3. Description of the clusters in the phylogenetic tree and phylotypes

Cluster	Salar de Huasco phylotypes	Closest GenBank entry (% similarity)	Closest cultured relatives (% similarity)	Habitat of closest relative	References
A	H1w-93	*Limnothrix* sp. CENA 110 (EF088338) (91%)		Waste stabilization pond, Brazil	GenBank information
B	H4s-42, H1w-72	Uncultured cyanobacterium clone A206 (DQ181671) (92–96%)	*Geitlerinema carotinosum* AICB 37 (AY423710) (92–95%)	Microbial mat, Lake Ace, Vestfold Hills, Antarctica	Taton *et al*. (2006a)
C	H1w-15	*Phormidium inundatum* SAG 79.79 (AM398801) (98%)		Thermal water, France	Marquardt & Palinska (2007)
	H1s-30, H1w-77, H1s-79, H1s-38	*Phormidium* sp. ETS-05 (AJ548503) (93–99%)		Thermal mud, Euganean thermal springs, Italy	Berrini *et al*. (2004)
	H0w-44, H0w-51	Uncultured Antarctic cyanobacterium clone Fr147 (AY151731) (96–99%)	*Phormidium uncinatum* SAG 81.79 (AM398780) (93%)	Microbial mat, Lake Fryxell, McMurdo Dry Valleys, Antarctica	Taton *et al*. (2003)
	H0w-87	Clone 173-2 (AJ871976) (97%)	*Microcoleus vaginatus* PCC 9802 (97%)	Biological soil crust, Colorado Plateau, USA	Gundlapally & Garcia-Pichel (2006)
	H0w-63, H0w-79, H0w-1	Uncultured cyanobacterium clone G1-1_9 (EF438215) (96–99%)	*Phormidium* sp. NIVA-CYA 203 (Z82792) (96–99%)	Epilithon, Douglas River, Ireland	GenBank information
	H1s-3	*Phormidium* cf. terebriformis KR2003/25 (AY575936) (96%)		Hot spring, Lake Bogoria, Kenya	Ballot *et al*. (2004)
	H1w-20	*Phormidium pseudopristleyi* ANT.ACEV5.3 (AY493600) (98%)		Microbial mat, Lake Ace, Vestfold Hills, Antarctica	Taton *et al*. (2006b)
	H4w-78, H4w-28, H4w-90	*Phormidium* sp. UTCC 487 (AF218376) (96–98%)		Canada, Artic	Casamatta *et al*. (2005)
D	H0s-1	Uncultured cyanobacterium clone SepB-17 (EF032663) (97%)	*Chamaesiphon subglobosus* PCC 7430 (AY170472) (97%)	River biofilm, Cloghoge River, Ireland	GenBank information
	H1w-7	*Nodularia spumigena* strain NSLA02A4 (AF268008) (93%)		Lake Alexandrina, SA, Australia	Moffitt *et al*. (2001)
E	H4s-37	*Aphanizomenon* cf. gracile 271 (AJ293125) (97%)		Lake Norre, Denmark	Gugger *et al*. (2002)
	H4s-56	*Anabaena cylindrica* PCC 7122 (AF247592) (95%)		Japan	Beltran & Neilan (2000)
	H1s-29	*Cyanospira rippkae* (AY038036) (97%)		Soda lake Magady, Kenya	Iteman *et al*. (2002)
	H1w-78	*Tolypothrix* sp. PCC 7415 (AM230668) (97%)		Soil, greenhouse, Stockholm, Sweden	Sihvonen *et al*. (2007)
	H1w-18	*Nodularia spumigena* strain NSLA02A4 (AF268008) (99%)		Lake Alexandrina, SA, Australia	Moffitt *et al*. (2001)
	H1w-86	*Nodularia spumigena* strain BY1 (AF268004) (99%)		Baltic Sea	Moffitt *et al*. (2001)
	H1w-59	*Nostoc* sp. 8941 (AY742448) (97%)		Gunnera dentata, New Zealand	Svenning *et al*. (2005)
	H1s-24	*Calothrix* sp. BECID30 (AM230685) (94%)		Rock surface, Baltic Sea, Finland	Sihvonen *et al*. (2007)
F	H0s-57	Uncultured cyanobacterium clone TAF-A202 (AY038730) (92%)	*Dermocarpella* sp. PCC 7326 (AJ344559) (91%)	Epilithon, River Taff, UK	O'Sullivan *et al*. (2002)
	H0s-2, H0s-58, H0w-42, H0s-6	Uncultured cyanobacterium clone TAF-A202 (AY038730) (94–98%)	*Pleurocapsa* sp. CALU 1126 (DQ293994) (94–98%)	Epilithon, River Taff, UK	O'Sullivan *et al*. (2002)
G	H4w-85, H4w-67	Uncultured cyanobacterium clone SC3-19 (DQ289927) (95%)	*Gloeothece* sp. KO68DGA (AB067580) (95%)	Sediment, South Atlantic Bight	Hunter *et al*. (2006)

Table 3. Continued.

Cluster	Salar de Huasco phylotypes	Closest GenBank entry (% similarity)	Closest cultured relatives (% similarity)	Habitat of closest relative	References
H	H6w-40, H1s-69, H6w-77	Uncultured bacterium clone MSB-2E11 (EF125441) (92%)	*Symploca* sp. VP642c (AY032934) (91%)	Mangrove soil	GenBank information
	H1w-14, H1s-52, H1s-53	Uncultured bacterium clone MSB-2E11 (EF125441) (93%)	*Gloeothece membranacea* PCC 6501 (X78680) (91–92%)	Mangrove soil	GenBank information
	H1w-3	*Synechocystis* PCC6805 (AB041938) (97%)			GenBank information
	H1w-19	*Merismopedia glauca* B1448-1 (X94705) (95%)		Microbial mat, Norderney Island, Germany	Palinska *et al*. (1996)
I	H1w-4	*Gloeocapsa* sp. PCC 73106 (AF132784) (94%)			Turner *et al*. (1999)
	H1s-95, H1w-80	Uncultured cyanobacterium clone GPENV127 (DQ512831) (97-95%)	*Synechocystis* sp. PCC 6308 (AB039001) (97-95%)	Gorompani warm spring, Assam, India	GenBank information
	H1w-31	*Cyanobacterium stanieri* PCC 7202 (AM258981) (98%)		Microbial mat, Euganean thermal springs, Italy	GenBank information
J	H4s-45	*Oscillatoria* sp. CCAP 1459/26 (AY768396) (98%)			GenBank information
	H1w-44	*Halomicronema excentricum* str. TFEP1 (AF320093) (93%)		Microbial mat, Eilat artificial ponds, Israel	Abed *et al*. 2002
	H1w-5	*Leptolyngbya* sp. 0BB32S02 (AJ639894) (93%)		Bubano basin, Imola, Italy	Castiglioni *et al*. (2004)
	H4s-26, H1w-92	Uncultured cyanobacterium clone Ct-3-39 (AM177427) (93%)	*Halomicronema* sp. SCyano39 (DQ058860) (92%)	Coral reef sediments, Heron Island, Australia	GenBank information
	H1w-35	*Leptolyngbya nodulosa* UTEX 2910 (EF122600) (93%)		South China Sea	Li & Brand (2007)
	H1w-65	*Leptolyngbya* sp. CCMEE6011 (AY790838) (95%)		Travertine rock, Narrow Gauge Lower Terrace, Yellowstone National Park, USA	Norris & Castenholz (2006)
	H4s-20, H4s-33, H4s-19, H6w-1, H4s-24, H4s-15, H1w-13	*Leptolyngbya* sp. 0BB30S02 (AJ639892) (95–98%)		Bubano basin, Imola, Italy	Castiglioni *et al*. (2004)
	H1w-53	*Leptolyngbya antarctica* ANT.ACEV6.1 (AY493589) (98%)		Microbial mat, Lake Ace, Vestfold Hills, Antarctica	Taton *et al*. (2006b)
	H1w-1	*Oscillatoria* sp. CCMEE 416 (AM398781) (98%)		Marble Point, Antarctica	Marquardt & Palinska (2007)
	H4w-62, H1w-8, H4s-66	*Leptolyngbya* sp. 0BB24S04 (AJ639893) (97–98%)		Bubano basin, Imola, Italy	Castiglioni *et al*. (2004)
K	H1w-71	Uncultured cyanobacterium clone G1-1_58 (EF438248) (97%)	*Leptolyngbya* sp. 0BB19S12 (AJ639895) (90%)	Epilithon, Douglas River, Ireland	GenBank information
	H1w-82	*Leptolyngbya frigida* ANT.LH70.1 (AY493574) (99%)		Microbial mat, Lake Reid, Larsemann Hills, Antarctica	Taton *et al*. (2006b)
	H1w-79	Uncultured cyanobacterium clone RJ004 (DQ181705) (99%)	*Leptolyngbya antarctica* ANT.LH18.1 (AY493607) (99%)	Microbial mat, Lake Reid, Larsemann Hills, Antarctica	Taton *et al*. (2006a)
	H1w-27	Filamentous thermophilic cyanobacterium tBTRCCn 302 (DQ471445) (96%)	*Oscillatoria* sp. OH25 (AF317508) (96%)	Zerka Ma'in thermal springs, Jordan	GenBank information

Table 3. Continued.

Cluster	Salar de Huasco phylotypes	Closest GenBank entry (% similarity)	Closest cultured relatives (% similarity)	Habitat of closest relative	References
L	H4s-61	Uncultured cyanobacterium clone RJ037 (DQ181715) (93%)	*Leptolyngbya antarctica* ANT.FIRELIGHT.1 (AY493590) (92%)	Microbial mat, Lake Reid, Larsemann Hills, Antarctica	Taton *et al.* (2006a)
	H4s-31	Uncultured Antarctic cyanobacterium clone Fr285 (AY151759) (94%)	*Leptolyngbya antarctica* ANT.FIRELIGHT.1 (AY493590) (93%)	Microbial mat, Lake Fryxell, McMurdo Dry Valleys, Antarctica	Taton *et al.* (2003)
	H4s-18, H6w-73	*Leptolyngbya antarctica* ANT.FIRELIGHT.1 (AY493590) (97-99%)		Microbial mat, Lake Firelight, Bolingen Islands, Antarctica	Taton *et al.* (2006b)

Percent similarity with closest relatives and closest cultured relatives in GenBank are shown.

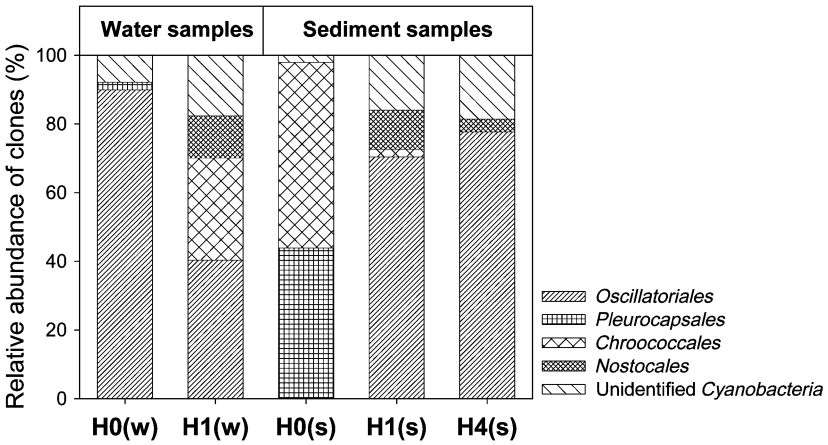

Fig. 3. Composition of the cyanobacterial 16S rRNA gene clone libraries from water (w) and sediment (s) samples of the sites H0, H1 and H4.

from site H0 water samples formed a separate group inside cluster C, with similarities between 96% and 99% with *Microcoleus vaginatus* and *Phormidium* sp. NIVA-CYA 203, both isolated from terrestrial environments from Arctic Norway (Rudi *et al.*, 1997). Sequences from Lake Fryxell in Antarctica (Taton *et al.*, 2003) and the clone 173-2 retrieved from soil crusts in the Colorado Plateau in USA (Gundlapally & Garcia-Pichel, 2006) are also part of this subcluster which has been described as Cluster I (Taton *et al.*, 2003). Sequences from this subcluster within cluster C have a particular 11-nucleotide insertion, first described for Antarctic and Artic species (Nadeau *et al.*, 2001), and also lately found in Antarctic clone libraries and in other nonpolar environments (Taton *et al.*, 2003). We found this insertion in the phylotypes H0w-1, H0w-87, H0w-79 and H0w-63, highlighting that this insertion can not be considered as a reliable indicator for endemism in Antarctic *Cyanobacteria*. The phylotype H1w-20 had 98% similarity with *Phormidium pseudopriestleyi* ANT.ACEV5.3, isolated from Lake Ace in Antarctica (Taton *et al.*, 2006b) and was included in a cluster related to saline environments (Taton *et al.*, 2006a). Three phylotypes of water samples from site H4 formed a separate group: clones H4w-78 and H4w-28 had 96–98% similarity with *Phormidium* sp. UTCC 487, isolated from benthic substrate in Canadian Arctic (Casamatta *et al.*, 2005). Clone H4w-90 had 99% similarity with *Phormidium* sp. OL S6, previously isolated from a microbial mat in the North Sea. Both *Phormidium* species formed one cluster (Marquardt & Palinska, 2007).

Cluster E was affiliated to the *Nostocales* and contained phylotypes from sites H1 and H4. Two sediment phylotypes from site H4 had $>95\%$ similarity with members of the *Nostocaceae*. Another set of phylotypes from H1 grouped together with *Nodularia*. Phylotypes H1w-18 and H1w-86 had 99% similarity with two strains of *Nodularia spumigena,* described as a planktonic, toxic, bloom-forming cyanobacterium with heterocysts and high 16S rRNA gene sequence similarity with other members of the genus ranging from 98.5% to 100% (Moffitt *et al.*, 2001; Lyra *et al.*, 2005), and with the clone A180 retrieved from microbial mats of Lake Ace in Antarctica (Taton *et al.*, 2006a). Phylotype H1w-59 had 97% similarity with *Nostoc* sp. 8941 isolated from

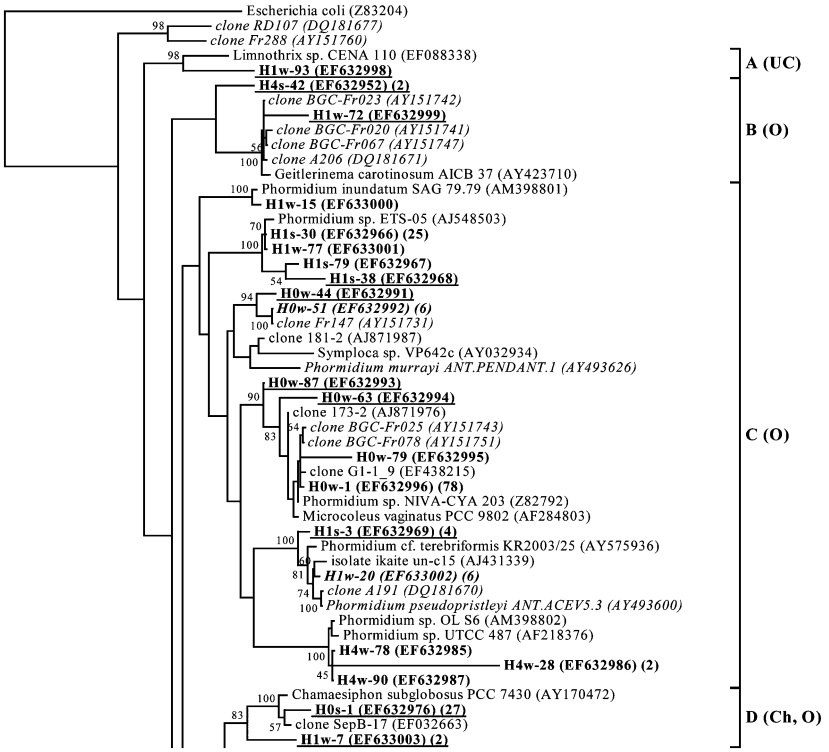

Fig. 4. Phylogenetic tree based on partial 16S rRNA gene sequences (c. 660 bp) calculated by maximum likelihood analysis. The scale bar represents 10% nucleotide sequence difference. Bootstrap values > 40% are shown. Clone sequences from this study are in bold and coded as follows (example of H0w-42): H, Salar de Huasco, site H0; w, water sample; 42, clone number. Underlined clones represent sequences with < 97.5% similarity to the closest relatives in BLAST. Clones in italics had > 98% similarity with their closest relatives retrieved from Antarctica. The number of clones in each phylotype is shown in brackets except for phylotypes with only one clone. Phylogenetic affiliations of the clusters are indicated as follows: UC, Unidentified *Cyanobacteria*; O, *Oscillatoriales*; N, *Nostocales*; P, *Pleurocapsales*; Ch, *Chroococcales*. *Escherichia coli* (Z83204) was used as outgroup.

Gunnera dentata in New Zealand (Svenning *et al.*, 2005). In the same cluster E, the phylotype H1s-24 showed 94% sequence similarity with *Calothrix* sp. ANT.LH52B.2, isolated from Lake Bruehwiler in Antarctica. This species was considered as a new phylotype (Taton *et al.*, 2006b).

Cluster F, affiliated to the *Pleurocapsales*, only contained phylotypes from site H0. Sequence similarity of the clones of this cluster ranged between 92% and 98% with clone TAF-A202 retrieved from sediment samples from epilithon of river Taff in the UK (O'Sullivan *et al.*, 2002). Clone H0w-42 had 98% similarity with *Pleurocapsa* sp. CALU 1126 (GenBank information).

Cluster I was affiliated to the *Chroococcales* and only included sequences from site H1. The phylotype H1w-31 had 98% similarity with *Cyanobacterium stanieri* PCC 6308 (GenBank information).

Cluster J included members of the *Oscillatoriales* and was formed with phylotypes from sites H1, H4 and H6 (Fig. 4, Table 3). Phylotype H4s-45 had 98% similarity with *Oscillatoria* sp. CCAP 1459/26 (GenBank information). Phylotype H1w-44 had 93% similarity with *Halomicronema excentricum* str. TFEP1, a new filamentous benthic genus isolated from microbial mats in artificial ponds from Eilat in Israel (Abed *et al.*, 2002). Three phylotypes (H1w-5, H4s-26, H1w-92) clustered together but at low similarity (< 93%), and their affiliation inside the *Oscillatoriales* was unclear. Phylotypes H1w-35 and H1w-65 clustered with *Leptolyngbya* sp. CCMEE6011 isolated from dry travertine rocks in the Yellowstone National Park in USA (Norris & Castenholz, 2006). Two phylotypes from site H1 water samples (H1w-53, H1w-1) exhibited < 98% similarity with Antarctic strains and sequences of clone libraries of 16S rRNA gene. Phylotypes H1w-13, H4w-62 and H1w-8 had 98% similarity with *Leptolyngbya* sp. 0BB24S02 and *Leptolyngbya* sp. 0BB24S04, isolated from Bubano basin in Imola, Italy (Castiglioni *et al.*, 2004). Another set of phylotypes exhibited similarity values lower than 97% with the strains described above.

Cluster K contained phylotypes of the water sample from site H1. Phylotype H1w-82 was 99% similar with *Leptolyngbya frigida* ANT.LH70.1, isolated from Lake Reid and considered as a new strain from Antarctica (Taton *et al.*, 2006b). Another phylotype, H1w-79, had 99% similarity with clone RJ004 from a cluster hitherto unique for Antarctic environments (Taton *et al.*, 2006a).

Cluster L was formed by four phylotypes retrieved of sediment samples from sites H4 and water samples from site H6. They grouped together with clones and one strain recovered from Antarctica. Phylotype H4s-18 had 99% similarity with *Leptolyngbya antarctica* ANT.FIRELIGHT.1 that was considered unique for Antarctica (Taton *et al.*, 2006b).

Biogeographical remarks

The current study has revealed elevated levels of microdiversity of cyanobacterial communities from different

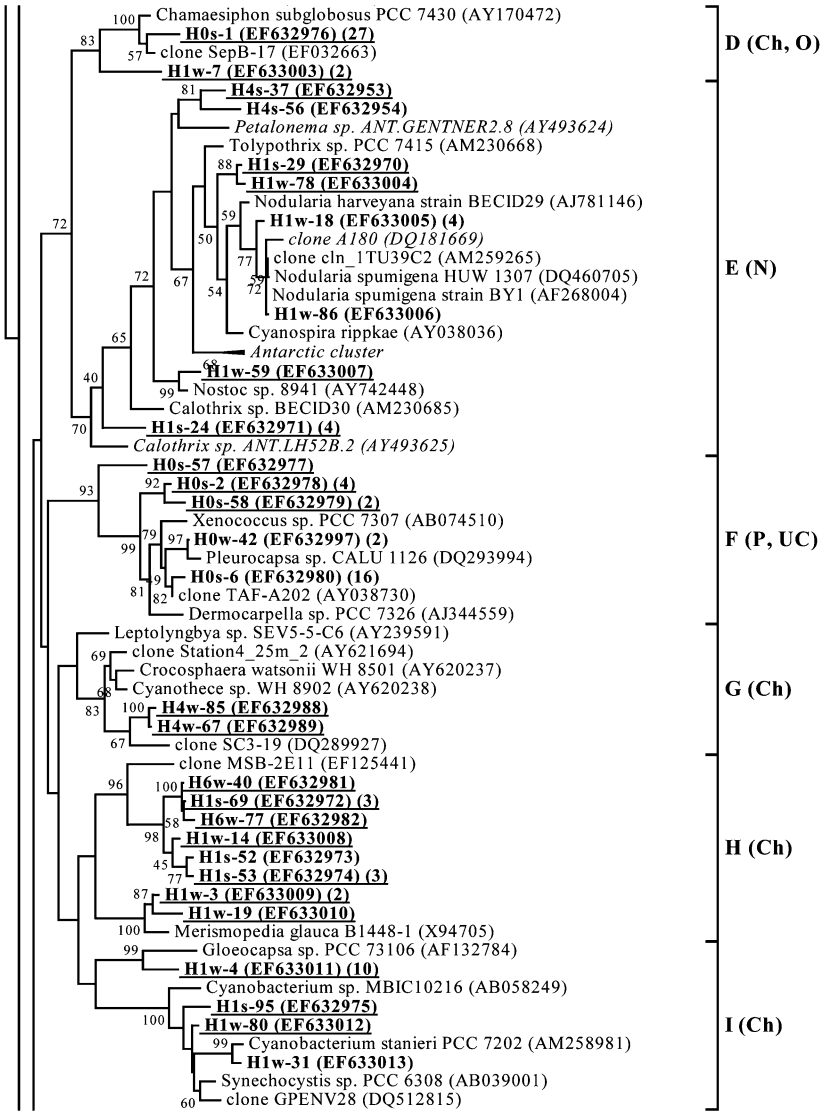

Fig. 4. Continued.

compartments of the Salar de Huasco, an almost unexplored water body in the Chilean Altiplano. This study also demonstrated that the apparent endemism of some clusters in Antarctic might simply reflect a lack of information regarding cyanobacterial diversity in remote areas. Our results indicated that 60–90% of sequences could be considered as new phylotypes (Fig. 2). However, we cannot assume that this phylotypes are endemic for Salar de Huasco based solely on 16S rRNA gene sequences and comparisons with information available in public databases. For example, when examined in September 2007 GenBank included > 32 000 cyanobacterial sequences, of which 1400 had a polar origin and *c.* 1300 were retrieved from hot springs (560 from Yellowstone National Park), compared with the 75 reported sequences from this study and 71 from Atacama Desert (Phoenix *et al.*, 2006; Warren-Rhodes *et al.*, 2006).

A particularly interesting finding of the present study is the presence of common phylotypes from both Antarctica and Salar de Huasco. It has been proposed that wind, birds and humans all have potential roles in the dispersion of algae (e.g. Broady, 1996). However, a study describing the cultured microbial diversity of different cold-remote areas (e.g. Antartica, Alps, Andes) suggests that micro-autotrophs (*Cyanobacteria* and algae) are not frequently transported over long distances with air-masses as previously supposed and may actually not be able to survive transport in this manner (Elster *et al.*, 2007). The endemic or cosmopolitan character of *Cyanobacteria* is still subject of debate. Komárek

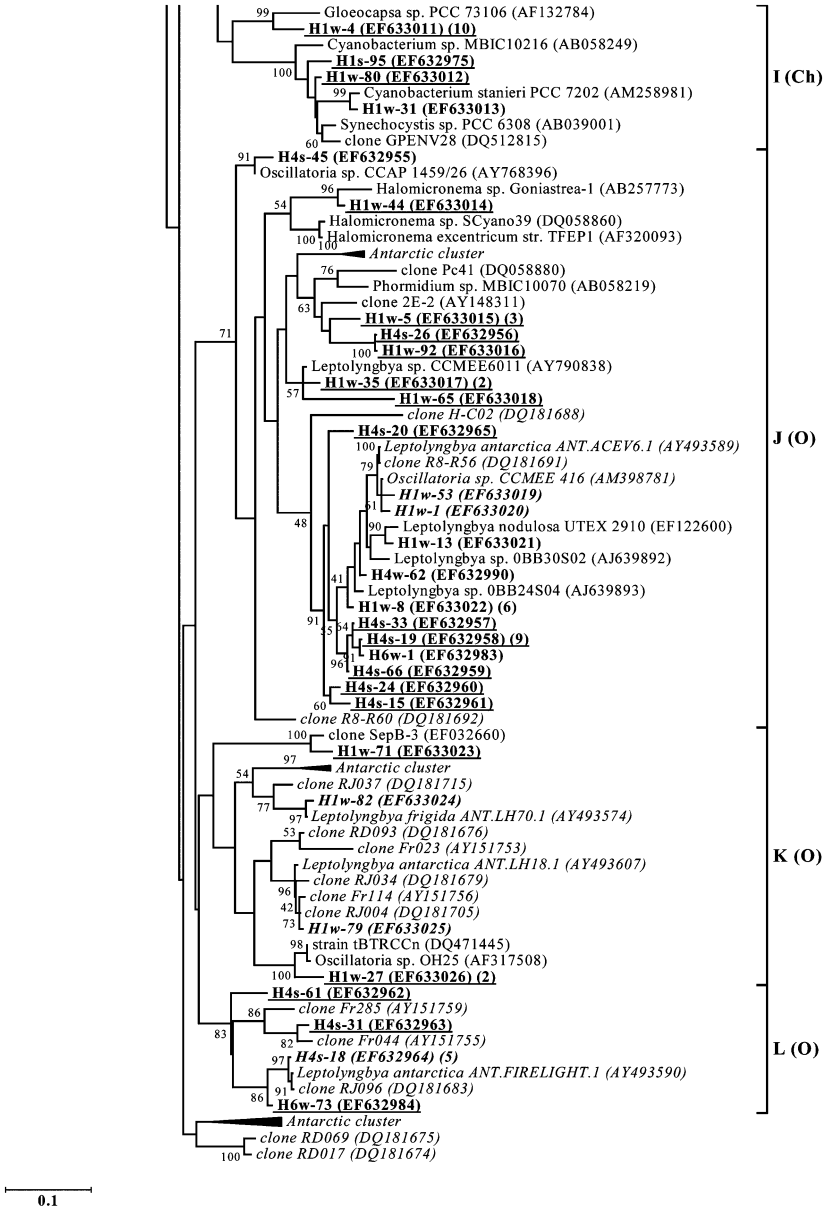

Fig. 4. Continued.

(1999) using cyanobacterial cultures found an elevated level of endemism in Antartica, however, other authors have reported opposite results (e.g. Vincent, 2000). Therefore there is a requirement for further studies of the mechanisms involved in cyanobacterial dispersion, but we can only speculate on the origin of the phylotypes present in Antarctica and Salar de Huasco.

Geographical endemism for prokaryotes has been reported recently in marine bacterioplankton using 16S rRNA gene sequences. Community structure in nine different marine locations worldwide was similar, but only 0.4% of the sequences were cosmopolitan (Pommier *et al.*, 2007). It is clear, that studies examining possible endemism in prokaryotes, requires a multilateral approach, including morphological and molecular aspects. Also, a consensus regarding species definitions for Bacteria and Archaea is necessary to established criteria for further microbial biodiversity patterns (e.g. Horner-Devine *et al.*, 2004; Prosser *et al.*, 2007).

Samples from Salar de Huasco were heterogeneous, and if other saline wetlands in the Altiplano would be included, this heterogeneity would increase notably. Taxon richness is typically positively related with ecosystem size (Horner-Devine *et al.*, 2004; Reche *et al.*, 2005). An examination of

Cyanobacteria communities across the Altiplano is likely to result in the description of more diverse cyanobacterial communities, and to provide insight into the possibly wide distribution of this group in extreme environments.

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